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Patentanmeldung Nr. | Patent application No. | Demande de brevet n°

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Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
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Preparation comprising a phytase, a phytate and an essential cation

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PREPARATION COMPRISING A PHYTASE, A PHYTATE AND AN ESSENTIAL CATION

Field of the invention

The invention relates to an edible preparation comprising a phytase, a phytate and an essential cation.

Background of the invention

The provision of minerals in a safe and efficacious way is a major challenge in human nutrition. Mineral deficiencies can have a severe impact on metabolic function and human health. Mineral deficiencies are severe, worldwide problems and existing methods of supplementation have failed to solve these. In some countries even an aggravation of the problem could be seen. A number of issues must be addressed (se for instance "The Mineral Fortification of Foods", R. Hurrell ed., Leatherhead Publishir Leatherhead, U.K., 1999) to tackle this problem. The main goal in reducing mineral deficiencies is to provide enough bioavailable minerals in a safe and efficacious way. The most bioavailable forms of mineral are often not suitable for inclusion into food, because of their bad taste, and their deleterious effect on the stability of the food. Furthermore although minerals are essential nutrients, serious negative effects may occur when the dosage is too high, due to oxidative damage or the formation of precipitates. Moreover, over-dosing of minerals ultimately promotes their proliferation into the environment, which is increasingly regarded as an undesirable phenomenon. And finally, the bioavailability of the minerals is strongly influenced by other compone of the food matrix and the ambient pH-value.

The first issue concerning the bad taste, and the deleterious effect of minerals on the stability of the food calls for a mineral preparation where the metal ions are shielded, that they have no effect on taste or food stability. Such preparations exist, for instanc the bisglycinates of iron, but their bioavailability is relatively low, and their price is higl The second and third issues relating to overdose and bioavailability are interrelated: t (often unknown) effect of the food matrix makes it difficult to assess the dosage level is required for a safe, yet efficacious supplementation of the mineral.

This is obviously true when the supplementation is performed in the food, but it is a true when the preparation is taken separately, because its uptake will still be depen on whether food is taken with, before or after the preparation, and on the kind of fo The presence or absence of food components in the gastrointestinal tract together the mineral will influence its uptake, either via direct interaction, or through their infl on parameters such as gastric pH value, emptying of the stomach, the secretion of salts, etc.

Phytate is a food component that is believed to have a particularly strong influence mineral and/or cation bioavailability (Jovanf et al., Food Sci. Tech. Int. (2001) 7:191 Lönnerdal, Int. J. Food Sci. Technol. (2002) 37:749-758). Phytate (or inositol-hexaki phosphate) is present in many foods of plant origin. The phytate salts of many nutritionally important cations are very poorly soluble. Hence, it is commonly known the art that the presence of phytate exerts a strong negative effect on the absorptio cations, such as iron (Fe), zinc (Zn), and calcium (Ca). Phytate can be hydrolyzed b enzyme phytase, which progressively splits off the phosphate groups of the inositol- hexakis-phosphate down to inositol-mono-phosphate (Zimmermann et al., Ernährun Umschau (2000) 47:423-427 and 472-476). Methods in the art have already attemp to reduce the phytate levels in food by applying phytase during food processing (Sir et al., Biotechnol. Adv. (1991) 122:145-161). This may be an externally added phyta the endogenous phytase activity of the foodstuff, or the phytase expressed by microorganisms during fermentation of the food. The result is a dephytinized food, usually without, or with a low, residual phytase activity. Examples of this technology been presented for soy protein isolate, for soy milk, for pea flour, for bread dough, a for cereals (Sandberg and Andlid, Int. J. Food Sci. Technol. (2002) 37:823-833). It h even been suggested that partially hydrolyzed phytate may aid in the solubilization mineral ions (Shen et al., Nutr. Biochem. (1998) 9:298-301).

This method has some disadvantages: (1) It requires a well-controlled step during th food processing, lasting long enough to break-down the phytate in an environment t allows access of the enzyme to the substrate; (2) Although it does reduce the phyta level of the foodstuff at hand, it does nothing about the interaction between minerals phytates present in the entire food matrix that is consumed.

This problem is partially overcome by adding active phytase to a foodstuff, to achiev hydrolysis of the phytate in the gut. This is the standard application method for phyta

for animal feed (Zimmermann et al., Ernährungs-Umschau (2000) 47:423-427 and 4
476) but it has also been shown to work in humans in cereal products (Sandberg et
J. Nutr. (1996) 126:476-480; Sandberg and Andlid, 2002). Recently, WO 02/05488
described the addition of *Aspergillus niger* phytase to milk before pasteurization.
Phytase was still active after pasteurization treatment of the milk. Therefore, milk ca
used as an effective delivery system of active phytase in food.

A dietary aid was described in WO 01/89317, which comprises an agent that assists
digestion. This agent can be a phytase. This dietary aid may comprise additional ag
such as other enzymes, minerals and/or phytate. The minerals are not bound to phy

A pharmaceutical composition was described in US 4,758,430 patent comprising ph
acid or its salts or hydrolysates as a medicine for the treatment of Alzheimer's disea
The phytate salt is a pharmaceutically acceptable salt such as salts with alkali metal
cations or salts with organic bases. The composition may be orally administrated. In
composition, phytic acid is the proposed active ingredient, in which phytase may be
present to hydrolyse to lower inositol derivates which could have specific
pharmacological effects. Huge quantities of phytate salts, compared to the average
intake of a population are to be ingested to be effective.

There is still a need for edible food products comprising the desired amount of availa
minerals without the negative impacts described above. The present invention provi
a method to deliver essential cations in an effective and safe way, independent of th
endogenous phytate levels of the food. This is achieved by means of a preparation,
providing simultaneously the essential cations, phytate and phytase. The phytase ac
as a liberating principle for the essential cations. Additionally, the nutritional benefits
phytate and partially hydrolyzed phytate are retained.

Detailed description of the invention

The present invention relates to a preparation comprising an active phytase, an esse
cation, and a phytate, wherein at least part of the essential cation is bound to phytat
Several methods of administration of the preparation are possible. For handiness
reasons, the most preferred method of administration is an oral administration, when

the preparation of the invention is an edible preparation. According to another yet preferred embodiment, the preparation of the invention is such that when it is prese the intestinal tract, essential cations are released from the phytate. This preferred embodiment is realized for example when a fungal phytase is used in the preparatio and/or when the phytase is coated to form a slow-release preparation. The preparat of the invention can be in the form of a dietary supplement, which can be ingested before, during or after the meal or which can be added in any food product preferabl the end of its processing. The food product comprising the preparation of the inventi hereafter named a fortified food product. The preparation of the invention and/or the fortified food product of the invention ensure sufficient bioavailability of the essential cations present therein regardless of the amount of phytate present in the food or in gastro-intestinal tract from previously or simultaneously consumed food.

In the context of this application, "a" means "at least one". Therefore, an active phyt means at least one active phytase, an essential cation at least one essential cation a phytate at least one phytate.

In the context of this invention, a phytase is an enzyme, which can convert phytate a phytic acid into phosphate and inositol phosphates. Any phytase activity may be use the present invention, such as for instance: 3-phytase EC 3.1.3.8., 6-Phytase EC 3.1.3.8., or 3,6-Phytase EC 3.1.3.8. According to another embodiment, a mix of sev phytases may be used. The phytase of the present invention may e.g. be derived fr microorganism such as a bacterium, a yeast, a fungus or from a plant. Preferably, th phytase is a fungal phytase. In contrast to phytases from plant origin, fungal phytase are also active under acidic conditions, having a high residual activity at pH= 2. This makes it possible to hydrolyze the phytate in the stomach, to prevent the subsequen formation of cation-phytate precipitates in the small intestine. More preferably, the phytase is from *Aspergillus niger*. A phytase from *Aspergillus niger* has already bee commercialized in animal feed and may also be used in the present invention as described in EP 0 420 358 A. The phytase from *A. niger* is commercialized under th trade name NATUPHOS™. This commercial phytase is available in liquid and solid formulations, and in concentrations of 5000 and 10000 FTU/g. 1 FTU is defined as t amount of enzyme, that liberates one micromole of phosphate per minute from 1Mm phytate at pH 5.5 at 37°C. The analytical method has been published (Engelen et al

AOAC, Int. 77:760-764 (1994)). Other phytases from *A. niger* may also be used in tl present invention such as the one described in KR 2001003164 A.

The gene encoding the phytase enzyme has been cloned and the phytase enzyme . been overexpressed in *Aspergillus niger*. *Aspergillus niger* is grown on industrial sc large fermentors allowing for the production of the enzyme. The enzyme is secreted large amounts by *Aspergillus niger*. Subsequently, the phytase is separated from the biomass in a series of filtration and ultrafiltration steps. The resulting concentrated ultrafiltrate is subsequently formulated into a stable granule or liquid, which may be u in the present invention. Inclusion of the enzyme in specific food products results in hydrolysis of phytate to inositol-rings bearing less phosphate groups and release of essential cations, that were associated with phytate. The availability of essential cat present in the food such as iron, calcium, magnesium, phosphorus, zinc, chromium, copper, manganese, molybdenum is therefore improved. At the same time, the protective potential health effect of partially hydrolyzed phytate or inositol is retained. According to another preferred embodiment, the phytase is not a native one, but a phytase enzyme that has been genetically modified in order to have improved prope such as heat stability and/or activity. Such phytases have already been described in following patent applications EP 0897 010, EP 0 897 985, WO 99/49022 or WO 00/43503. The genetically modified phytases that can be used in the preparation are limited to these ones.

In the context of the invention, an active phytase is an enzyme preparation capable to convert phytate and phytic acid into phosphate and inositol phosphates. The quantity active phytase present in the preparation of the invention has to be calculated in orde ensure that a majority of the essential cations bound to phytate would be released fro phytate. The needed amount of active phytase can be calculated taking into account t amount of phytate that will have to be hydrolyszed, the time in which this has to occur the pH-activity-profile of the active phytase chosen,the extent of hydrolysis one wants achieve and the identity of the essential cations bound to phytate. Several quantities c active phytase may be present in the preparation of the invention such as for example less than 1000 FTU phytase per gram phytate present in the preparation, or less than 500 FTU phytase per gram phytate present in the preparation, or less than 100 FTU phytase per gram phytate present in the preparation, or less than 50 FTU phytase pe gram phytate present in the preparation, or less than 20 FTU phytase per gram phytat

present in the preparation, or more than 1 FTU phytase per gram phytate present in preparation, or more than 5 FTU phytase per gram phytate present in the preparatio or more than 10 FTU phytase per gram phytate present in the preparation, or more 20 FTU phytase per gram phytate present in the preparation, or more than 50 FTU phytase per gram phytate present in the preparation, or more than 100 FTU phytase gram phytate present in the preparation. Several ranges of active phytase may be present in the preparation of the invention such as for example: between 1 and 100 FTU phytase per gram phytate present in the preparation, or between 1 and 600 FT phytase per gram phytate present in the preparation, or between 1 and 300 FTU ph per gram phytate present in the preparation, or between 10 and 100 FTU phytase p gram phytate present in the preparation. The preparation of the invention is in no wa limited to the ranges of phytase activity disclosed in this paragraph. If a higher or a phytase activity is needed for a specific application, the person skilled in the art wou know how to calculate the needed amount of phytase. A calculation example is give the examples.

The quantity of essential cation bound to phytate present in the preparation of the invention and the amount of preparation the fortified food product of the invention w comprise may be calculated in order to ensure that the amount of essential cations bound to phytate that would be released from it through the action of phytase would amount to a physiologically acceptable amount. The amount of preparation the fortif food product of the invention may comprise depends on several parameters such as intake of the essential cation that is recommended according to the normally used definitions such as Recommended Dietary Allowance (RDA), Adequate Intake (AI), Estimated Average Requirement (EAR)). Furthermore, the concentration is depende on the amount of essential cation that binds to the phytate. If the essential cation's valence is one (monovalent cation), the preparation comprises between 1 and 12 essential cations per phytate residue; if the essential cation's valence is two (bivalen cation), the preparation comprises between 1 and 6 essential cations per phytate residue; if the essential cation's valence is three, the preparation comprises betwee and 4 essential cations per phytate residue. Higher valences are not common in assimilated essential cations but are in no way incompatible with the invention. The concentration is also dependent on the molecular weight of the essential cation itsel Also the phytase content can vary with the application used. Several quantities of

essential cations may be present in the preparation of the invention such as for exa more than 1 g essential cation bound to phytate and less than 99 g phytate per 100 essential cation bound to phytate, or more than 5 g essential cation bound to phytat and less than 95 g phytate per 100g of essential cation bound to phytate, or more th 10 g essential cation bound to phytate and less than 90 g phytate per 100g of esse cation bound to phytate, or more than 20 g essential cation bound to phytate and le than 80 g phytate per 100g of essential cation bound to phytate, or more than 30 g essential cation bound to phytate and less than 70 g phytate per 100g of essential c bound to phytate, or more than 40 g essential cation bound to phytate and less than phytate per 100g of essential cation bound to phytate, or less than 50 g essential ca bound to phytate and more than 50 g phytate per 100g of essential cation bound to phytate, or less than 40 g essential cation bound to phytate and more than 60 g phy per 100g of essential cation bound to phytate, or less than 30 g essential cation bou phytate and more than 70 g phytate per 100g of essential cation bound to phytate, o less than 20 g essential cation bound to phytate and more than 80 g phytate per 10 essential cation bound to phytate, or less than 10 g essential cation bound to phytat and more than 90 g phytate per 100g of essential cation bound to phytate. Several ranges of amount of essential cations may be present in the preparation of the inven such as for example between 1 and 50 g essential cation bound to phytate and betw 50 and 99 g phytate per 100g of essential cation bound to phytate, or between 10 ar 45 g essential cation bound to phytate and between 55 and 90 g phytate per 100g o essential cation bound to phytate, or between 20 and 40 g essential cation bound to phytate and between 60 and 80 g phytate per 100g of essential cation bound to phyt or between 25 and 35 g essential cation bound to phytate and between 65 and 75 g phytate per 100g of essential cation bound to phytate.

The fortified food product of the invention is in no way limited to the examples of am of preparation given in the above paragraph. If a higher or a lower preparation amou needed for a specific food product as a result of an adjusted RDA, and/or a change i the quantity of essential cations bound to phytate and/or the molecular weight of the essential cations, the person skilled in the art would know how to calculate the need amount of preparation. Calculation examples are given in the Examples.

To deliver appropriate amounts of essential cation to the intestinal tract of a human being, eating the preparation of the invention or a fortified food product comprising t preparation of the invention would preferably amount to eat not more than between and 20 mg of phytate/kg of body weight / day. This amount of phytate is in the same order as the amount of phytate normally ingested in a normal daily diet. Preferably, amount of phytate ingested is ranged between 1 and 15 mg/kg body weight/day, mo preferably between 1 and 10 mg/kg body weight/day. The quantities of phytate inge by eating the preparation or the fortified food product of the invention are in no way limited to the ranges disclosed in this paragraph.

An essential cation is a cation, which is needed for human physiological processes as the cations of magnesium, iron, zinc, calcium, cobalt, molybdenum, manganese, chromium, copper. A deficiency in an essential cation could lead to severe diseases example, a deficiency in iron could lead to iron deficiency anemia. A deficiency in calcium could lead to osteoporosis. A deficiency in zinc could lead to a lowered imm response and a reduction in linear growth. A deficiency in zinc or iron during pregna could lead to impaired brain development of the foetus. According to another prefer embodiment an essential cation is a metal ion. A metal is a chemical element that ir general is characterized by the ability to form cations by loss of one or more electro from each atom.

The preparation according to the invention can be made with any essential cation. Preferably, the essential cation does not inhibit the phytase activity to an extent that will no longer be active in the intestinal tract. Preferred essential cations are the cati of magnesium, iron, zinc, calcium, cobalt, molybdenum, manganese, chromium, co or a combination thereof.

In the preparation according to the invention, the essential cations present are such at least part of them is bound to phytate as their phytate salt. In that way, the availal of the added essential cation is guaranteed by the presence of phytase. The prepar of essential cations bound to phytate has been described previously, for instance in Vasca et al., Anal. Bioanal. Chem. (2002) 374:173-178. At least part of the essentia cations means at least 30% of the essential cations, preferably at least 40%, more preferably at least 50%, most preferably at least 60% and even most preferably at le

90%. Even most preferably, no detectable free essential cation is present in the preparation of the invention, as measured in the following standard assay. This assa uses the low solubility of the essential cations bound to phytate compared to other s of essential cations. For instance, iron phosphate will dissolve at pH= 2, whereas iro phytate will not. In strong acids, such as 30% HCl, both salts will dissolve. In genera most, if not all, relevant essential cation salts will be more soluble at a given pH valu than the phytate salt of the same essential cation.

Alternatively, the assay method used can be the following: a powdered preparation i analyzed via powder X-ray diffractometry. The powder is subjected to x-rays and diffracted x-rays can be examined via the use of a suitable x-ray recorder, which car x-ray film, one dimensional x-ray detector, two dimensional area detectors or an electronic x-ray detector or scintillator. In principle x-ray analysis is not limited to the powder form. For example the material to be analyzed may be a number of loose crystals lumped together, twinned crystals or single crystals. Through such methods spacial relation of the essential cations and the inositol-phosphate rings may be established.

Prolonged calcium-deficiency can lead to severe diseases. It can be a factor in the o and/or progression of osteoporosis. Calcium-deficiency may be prevented by administering the preparation of the invention, wherein the essential cation is calciun According to another yet preferred embodiment, when calcium is the essential catior vitamin D is also present to ensure a maximum quantitative uptake of calcium, as vit D, in the form of 1,25 $(OH)D_3$, stimulates calcium transport across the intestinal cells inducing the production of a calcium binding protein (CBP). Vitamin D is present in amounts ranging from 1 – 10 microgram/day. Preferably the amount is between 2- 8 micrograms/day, most preferably the amount is between 2.5 and 5 micrograms/day. According to another yet preferred embodiment, the preparation comprises as esser cation calcium and at least one of the following essential cations: magnesium, iron, z cobalt, molybdenum, manganese, chromium, copper. Preferably, when the essential cation is calcium, magnesium is also present as essential cation. More preferably, w the essential cation is calcium, magnesium is present as essential cation as well as vitamin D. This specific preparation is specifically effective as bone mineral formula t optimal bone mineralisation. The combination of these three factors, together with th

phosphate groups derived form the hydrolysed phytate, provides a complete bone mineral formula.

Magnesium aids in optimal bone mineralisation and/or aids in the optimalisation of hundreds of enzymatic reactions for which magnesium is a cofactor. Furthermore, magnesium plays an important role in protein and nuclei acid synthesis and has a stabilising and protecting effect on membranes. Magnesium is also considered to be essential in maintaining Ca, K and Na homeostasis. Magnesium-deficiency may be prevented by administering the preparation of the invention, wherein the essential ca is magnesium. According to another yet preferred embodiment, the preparation comprises as essential cation magnesium and at least one of the following essential cations: calcium, iron, zinc, cobalt, molybdenum, manganese, chromium, copper.

Prolonged iron-deficiency can lead to reduce physical work capacity and productivity immuno-competence. Iron deficiency can also lead to iron deficiency anemia. Furthermore, reducing iron deficiency during pregnancy reduces the prevalence of prenatal mortality, low birth weight and fetal wastage and aids in improving cognitive functions. Iron-deficiency may be prevented by administering the preparation of the invention, wherein the essential cation is iron. Such a preparation does not have the drawbacks of other known iron supplements such as bad taste. Furthermore, there i reduced risk of iron overdose, since a specific quantity of iron would be delivered in intestinal tract. This can be achieved by tuning the phytase activity in the preparation the desired amount of phytate-bound iron to be released. Finally, for such a prepara it is very advantageous to adapt the formulation of the preparation to obtain an in-sit delivery system for the essential cation (also named a slow-release preparation). Th can be achieved by choosing the formulation so that the phytase would not be active the stomach but only later on in the intestinal tract. According to another yet preferre embodiment, when iron is the essential cation, vitamin C is also present to ensure a maximum quantitative uptake of iron. Vitamin C enhances the uptake of non-haem i in the intestine. Vitamin C is preferably present in amounts ranging from 5-95 milligram/day. More preferably the amount is between 15 - 70 milligram/day, most preferably the amount is between 25 - 60 milligram/day.

According to another yet preferred embodiment, the preparation comprises as essen cation iron and at least one of the following essential cations: calcium, magnesium, zi cobalt, molybdenum, manganese, chromium, copper.

Prolonged zinc-deficiency can lead to severe diseases such as immunodeficiency an diminished linear growth in children. Furthermore, it can lead to skeletal abnormalitie and impaired reproductive capacity. Zinc-deficiencies may be prevented by administering the preparation of the invention, wherein the essential cation is zinc. According to another yet preferred embodiment, the preparation comprises as essen cation zinc and at least one of the following essential cations: calcium, magnesium, i cobalt, molybdenum, manganese, chromium, copper.

Cobalt-deficiency may be prevented by administering the preparation of the inventior wherein the essential cation is cobalt. According to another yet preferred embodimer the preparation comprises as essential cation cobalt and at least one of the following essential cations: calcium, magnesium, iron, zinc, molybdenum, manganese, chromi copper.

As molybdenum is a cofactor in three oxidases enzymes, prolonged molybdenum- deficiency can lead to disturbed metabolic processes such as abnormal sulfur metabolism and developemental and neurological abnormalities. Molybdenum-defici may be prevented by administering the preparation of the invention, wherein the essential cation is molybdenum. According to another yet preferred embodiment, the preparation comprises as essential cation molybdenum and at least one of the follow essential cations: calcium, magnesium, iron, zinc, cobalt, manganese, chromium, copper.

Prolonged manganese-deficiency could lead to effects such as growth retardation ar impaired skeletal development in the fetus. Manganese-deficiency may be preventec administering the preparation of the invention, wherein the essential cation is manganese. According to another yet preferred embodiment, the preparation compri as essential cation and at least one of the following essential cations: calcium, magnesium, iron, zinc, molybdenum, cobalt, chromium, copper.

Prolonged chromiun-deficiency can lead to severe diseases such as glucose intolerance. Chromium-deficiency may be prevented by administering the preparati the invention, wherein the essential cation is chromium. According to another yet preferred embodiment, the preparation comprises as essential cation chromium anc least one of the following essential cations: calcium, magnesium, iron, zinc, molybdenum, cobalt, manganese, copper.

Prolonged copper-deficiency can lead to severe diseases such as anemia with alter iron metabolism and bone marrow changes, impaired immunity with low neutrophil count, skeletal changes with fractures and osteoporosis, hernia and tortuous dilated blood vessels from collagen and elastin cross-linking effects, hair and skin de-pigmentation with steely uncrimped hair. Copper-deficiency may be prevented by administering the preparation of the invention, wherein the essential cation is coppe According to another yet preferred embodiment, the preparation comprises as esse cation copper and at least one of the following essential cations: calcium, magnesiu iron, zinc, molybdenum, cobalt, manganese, chromium.

Additional components may be included in these formulations, such as chelating ag to keep the metal ions in solution, and antioxidants, to avoid any residual oxidative stress.

The preparation comprises a phytate, an essential cation, wherein at least part of th essential cation is bound to phytate and a phytase. The physical form of single components may be a liquid, a solid, or a two-phase system (solid in liquid). This ap both to the phytate and the phytase.

Phytate can be in a solid form, when the pure essential cations are bound to phytate (with various essential cation contents, the other counter-ions being hydrogen), or c be present in the form of mixed salts, wherein more than one essential cation is pre as counter-ion for the phytate. As an example: using Fe(III), Na+ and H+ as counter it is possible to make a Fe-phytate with any desired pH-value (upon mixing with wat and Fe-content. The solid may be made by spontaneous crystallization (which usua gives very fine powder), or by evaporation (which may give a coarser powder).

Liquid forms would be the dissolved essential cation bound to phytate in water or bu
This could be achieved with a mixed salt as just described, at the cost of a relatively
content of the essential mineral.

Mixed forms of phytate would be suspensions or dispersions of the phytate in water
aqueous solutions, for instance a buffer. To make it possible to handle such mixture
stabilizer can be added. A good example is xanthan-gum, which forms a gel when ir
(preventing sedimentation of the phytate), but which becomes liquid when poured
(allowing dosage of the mixture). This kind of formulation may be useful, because th
use of solids is sometimes impractical, for instance when the aim is to coat a solid fc

The phytase may be present in solid forms such as powder (f.i. spray dried) or
granulated forms. Example of food-grade granulation process is to mix a liquid phyta
preparation with starch, add moisture to create a dough, extrude the dough, and dry.
Phytase can be present in a liquid form such as a stabilized concentrated filtrate. We
known food-grade stabilizers can be used for this purpose, such as glycerol or sorbit

For preparing the preparation of the invention, phytate bound to the essential cation
the phytase may be simply mixed if they are both solid. If phytate bound to the esser
cation and the phytase are in a liquid form, it is impossible to mix them, because the
enzyme would already start breaking down the phytate in solution. To solve this prob
one could absorb both the phytate bound to essential cation and the phytase to a foc
component (but different ones). For instance: oat flakes with absorbed phytase and
wheat bran with absorbed phytate bound to essential cation could be used to prepare
the preparation of the invention and/or a fortified food product comprising a stable ce
food comprising the preparation of the invention. If either phytate bound to an essenti
cation is liquid and the phytase is solid, or vice versa, the phytate bound to an essent
cation must be protected. This may be done by encapsulation of the solid partner, to
separate it from the liquid fraction, which may then be absorbed by the coating, or by
absorption of the liquid partner by another solid.

The choice of the formulation may influence the application: normally, the phytate will
dissolve in the stomach, and the phytase will attack the phytate there. But if either of t
two is encapsulated in a way to be released in the gut, they will only come together at
the stomach, and this could be considered to be an in-situ-delivery concept (Protein
Formulation and delivery, Ed. E.J. McNally, Marcel Dekker Inc, New York, 2000, ISE

0-8247-7883-9; Handbook of Pharmaceutical Controlled Release Technology, Ed. D Wise, Marcel Dekker Inc. New York, 2000, ISBN 0-8247-0369-3). An in-situ-delivery formulation for lipase has already been described in European patent application EP 913468 A. The whole content of this patent application is incorporated in the present patent application. It means that the coating described for lipase in EP913468 A can applied in the present invention to prepare a coating for phytase and/or phytate bou essential cations.

There are many methods known to the skilled person for preparing phytate bound to essential cation. Phytate bound to an essential cation can be prepared chemically as described in Vasca et al., Anal. Bioanal. Chem. (2002) 374:173-178. Other publicati already described how to chemically prepare Ca-phytate (US4070493, EP575550B) Zn-phytate (ES2007238A). Alternatively, phytate bound to an essential cation can be prepared starting from organic materials. For example, Fe-phytate could be prepare starting from barley phytin (A.B. Stockholms Bryggerier, Swed., Congr. Intern. Inds. Fermentation, Confs. Et communs. (1947) 88-93), Ca-phytate from rice bran (CN1165142A, EP409494B), from distillery grains (CN1050541A) or from steep wat (US 3410929), Zn-phytate from plant seeds (CN1055556A). Phytate bound to an essential cation may also be prepared from wheat bran. In this case, an acid extract made of the wheat bran. Subsequently, the acid extract is titrated with an alkaline m salt comprising the desired essential cation. Alternatively, the acid phytate extract m first be neutralized, using a strong base, and subsequently soluble salts of one or m essential cations may be added, to achieve precipitation of phytate-bound essential cations. It will be understood that this precipitation may be performed at different an pH values, and that this will influence the composition of the resulting essential catio phytate.

The preparation of the invention may be added to any food or drink product for huma consumption. Preferably, preparation, storage or subsequent use of the food produc does not involve conditions incompatible with phytase activity. In a preferred embodiment, the preparation of the food product does not involve long heat treatme above 100°C and/or the food product does not need to be kept chilled prior to be us and/or does not need to be kept frozen prior to be used. Alternatively, the preparatic

can be added to the food product at the end of its processing. In that case, preferably only the storage conditions are to be compatible with phytase activity. The food product may be a dry food product. A dry food product is a food product, which may comprise less than 30% water w/w, or less than 25%, or less than 20%, or less than 16%.

According to a preferred embodiment, the dry food product comprises cereal. Cereal an interesting food product since it comprises high amount of phytate. More preferably the dry food product comprises muesli, rice or pasta or a combination thereof. According to a most preferred embodiment, the cereal is a cereal, which has a phytate content of more than 0.2 mg / 100 g cereal. More preferably, the phytate content is more than 0 mg / 100 g cereal. According to another most preferred embodiment, the dry food product is a cereal bar. According to another preferred embodiment, the dry food product is bread, cake, pastry, flour or a cracker.

According to another preferred embodiment, the product is a drink product. The drink product may be typically formulated for human consumption in terms of taste and look. The drink product may be a flavored drink and may be carbonated. Typically, the drink product is one, which is kept chilled or refrigerated. According to another preferred embodiment, the drink product is a milk. Preferably the milk is cow's milk or soymilk. More preferably, the cow's milk is pasteurized cow 's milk. In that case, the preparation can be added even before the pasteurization step (WO 02/054881); the phytase activity would not be affected by the pasteurization treatment.

According to another preferred embodiment, the food product comprises or is made of milk comprising the preparation of the invention such as cheese, yogurts, milk shakes, creams and desserts, or such as tofu or other soymilk-derived products.

According to another yet preferred embodiment, the food product is a condiment as defined below.

The preparation and fortified food product of the invention can be given to healthy individuals, as part of their normal diet. However, they also could be given to those suffering from mineral deficiencies and may be given to treat, alleviate, or prevent such deficiencies. They may be given to individuals suffering from iron deficiency anemia, calcium, zinc deficiency. They may also be given as part of the diet of pregnant women or women that recently gave birth.

The present invention further relates to condiments comprising an active phytase. This means that the condiment comprises at the end of its processing a phytase at a

concentration of from 5,000 to 1,000,000 FTU/kg, preferably from 10,000 to 500,00C FTU/kg, and most preferably from 50,000 to 150,000 FTU/kg. We surprisingly found phytase activity is very stable in this type of food products even after prolonged stora at room temperature.

A condiment is a food product usually pungent, acid, salty, or spicy added to or serv with food to enhance its flavor or to give added flavor. The condiment can be of natu origin such as pepper, vinegar, mustard. Alternatively, it could be of any various com compositions being a flavor enhancers such as curry chili powder, chili sauce, fish sauce, pickles, ketchup, tomato sauce, soy sauce. The condiment may also be a m or less pure chemical substance commonly used in food, such as table salt or monosodium glutamate (MSG). Preferably, the condiment is soy sauce or tomato sa According to another preferred embodiment, the condiment is supplemented with essential cation such as magnesium, iron, zinc, calcium, cobalt, molybdenum, manganese, chromium, copper or a combination thereof. In that way, the availability the added minerals is guaranteed by the presence of phytase.

The phytase may be present in solid or liquid forms as described above. The desire quantity of phytase is subsequently added to the condiment.

A condiment is an attractive delivery vehicle for phytase. It usually does not contain significant amount of phytate it-self. It is eaten by many people together with various types of food, among which are phytate and/or essential cation rich foods such as (whole grain) pasta, (whole grain) rice, whole wheat bread.

The present invention further relates to cereal food product comprising an active phytase. The cereal food product comprises at the end of its processing a phytase a concentration of from 150 to 30,000 FTU/kg, preferably from 300 to 15,000 FTU/kg, most preferably from 1,500 to 4,500 FTU/kg. We surprisingly found that phytase acti is very stable in this type of food products even after prolonged storage at room temperature.

More preferably, the cereal food product is muesli, rice or pasta or a combination thereof. According to a most preferred embodiment, the cereal food product is a cere which has a phytate content of more than 0.2 mg / 100 g cereal. More preferably, the cereal food product is a cereal which has a phytate content of more than 0.5 mg / 10 cereal. According to another most preferred embodiment, the cereal food product is

cereal bar. According to another preferred embodiment, the cereal food product is bre cake, pastry, flour or a cracker.

According to another preferred embodiment, the cereal food product is supplemented with essential cation such as magnesium, iron, zinc, calcium, cobalt, molybdenum, manganese, chromium, copper or a combination thereof. Cereals may comprise huge amount of phytate. Supplementing cereals with essential cations is not always an effective delivery vehicle of essential cation to the person who would eat the cereals, since the essential cations may bind to the endogenous phytate contained in the cere In the cereals of the invention, the availability of the added essential cation is guarant by the presence of phytase.

The phytase may be present in solid or in liquid forms as described above. The desire quantity of phytase is subsequently added to the cereals.

Cereal food products are an attractive delivery vehicle for phytase. Adding active phytase, preferably at the end of the cereal's processing is a way to deliver enough phytase to work in the intestinal tract when the cereals would be eaten with a meal. Cereal food products are therefore also an attractive delivery vehicle for phytase to reduce the phytate delivered by other components in the food matrix, in addition to the phytate present in the cereal it-self and thereby improve the mineral bioavailability fro the minerals present in the entire food matrix.

The present invention further relates to soymilk comprising an active phytase. This means that the soymilk comprises at the end of its processing a phytase at a concentration of from 500 to 20,000 FTU/kg, preferably from 1,000 to 10,000 FTU/kg, and most preferably from 2,000 to 5,000 FTU/kg. We surprisingly found that phytase activity is very stable in this type of food products even after a pasteurization treatmer Soymilk is defined as a mixture of soybean-derived solids and water. This may be prepared by a direct aqueous extraction of processed soybeans. Alternatively, the soybeans may first be fractionated, for instance to obtain a soybean meal or a partly purified soybean protein isolate, and subsequently the fractions may be mixed with w to obtain the soymilk. Various additives may further be added to this milk, such as sources of Calcium and phosphate, flavoring compounds, salt, sugar, etc. Many methods to prepare soybean fractions and soymilk are described in the book Soybea Utilization (Snyder HE and Kwon TW, Van Nostrand Reinhold Comp, New York, 198 According to another preferred embodiment, the soymilk is supplemented with an

essential cation such as magnesium, iron, zinc, calcium, cobalt, molybdenum, manganese, chromium, copper or a combination thereof. In that, way, the availabili the added essential cation is guaranteed by the presence of phytase

The phytase may be present in solid or liquid forms as defined above. The desired quantity of phytase may be added at the beginning of the soymilk's processing or m be subsequently added to the soymilk at the end of its processing.

In contrast to cow's milk, soymilk comprises high amounts of phytate. Adding active phytase, preferably at the beginning of the soymilk's processing is a way to diminis phytate content. At the same time, there would be enough phytase to work in the intestinal tract when the soymilk would be consumed with a phytate rich meal. Soyn therefore also an attractive delivery vehicle for phytase to reduce the phytate delive by other components in the food matrix and thereby improve the mineral bioavailabi from the minerals present in the entire food matrix. Adding active phytase, preferabl the end of the soymilk's processing is a way to deliver phytase to reduce the phytate delivered by other components in the food matrix and thereby improve the mineral bioavailability from the minerals present in the entire food matrix.

The invention will further be illustrated by the following examples.

Examples

Example 1:

Calculation of the amount of active phytase present in the preparation of the invention

For instance, 1 FTU will liberate 60 micromoles of phosphate per hour at pH= 5.5 and 37°C . If one would want to achieve 50% hydrolysis of phytate, corresponding to the release of 3 phosphate groups per phytate molecule, in 1 hour, 1 FTU would be sufficient for 20 micromoles of phytate (under optimal conditions). If we would use NATUPHOS™ , which has residual activity at characteristic stomach pH-values of ab 50% of its optimal activity, this number would drop to 10 micromoles, corresponding t 6.6 mg of phytic acid. This is equivalent to 150 FTU per gram phytic acid. The dosage per gram of essential cation bound to phytate will be less, depending on the mass rati of the cations and the phytic acid. If the cations would consitute 1/3 of the preparation the dosage would be 100 FTU per gram of essential cation bound phytate.

Example 2:

Calculation of the amount of essential cation present in the preparation of the invention and the amount of preparation the fortified food product of the invention may comprise

We below calculated the amount of preparation the fortified food product of the inven may comprise for two examples of essential cations: calcium and chromium. Calcium has been chosen as example of essential cation, since it is the essential cation with t highest AI, whereas chromium is a trace element. The following assumptions are us for calcium:

- The AI for women aged 19 - 30 years is 1000 mg/day (Dietary Reference int for Calcium, Phosphorus, Magnesium, Vitamin D and Fluoride. Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. Food ar

Nutrition Board, Institute of Medicine The National Academy Press Washing D.C., 1997, ISBN 0-309-06350-7 p 71-145)

- the phytase content of the preparation is 10 % of the phytate content
- in the preparation used, 3 mol of Ca have been bound to 1 mol of phytate.

The ranges are calculated in order of preference according to

10 -100 % AI

15-75 % AI

25- 50 % AI

Under these conditions, the following preferred ranges are found: preferably fortified food product comprises the preparation of the invention in a concentration, which is ranged between 698 - 6980 milligrams per 100 gram fortified food product, more preferably between 1047 - 5234 milligrams per 1 grams of fortified food product and most preferably between 1745 - 3490 milligrams per 100 gram of fortified food product.

The following assumptions are used for chromium:

- The AI for women aged 19 - 30 years is 25 microgram/day (Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc. Standing Committee on the Scientific Evaluation of Diet Reference Intakes. Food and Nutrition Board, Institute of Medicine The National Academy Press Washington D.C., 2001 ISBN 0-309-07279-4 p.197-223).
- the phytase content of the preparation is 10 % of the phytate content
- in the preparation used, 2 mol of Cr have been bound to 1 mol of phytate

The ranges are calculated in order of preference according to

10 -100 % AI

15-75 % AI

25- 50 % AI

Under these conditions, the following preferred ranges are found: preferably the fort food product comprises the preparation of the invention in a concentration, which is ranged between 19 - 199 micrograms per 100 grams of fortified food product, more

preferably between 29 - 145 micrograms per 100 grams of fortified food product and most preferable between 49 - 100 micrograms per 100 gram of fortified food product

Example 3:

Preparation of metal phytates (PA) and characterization thereof.

Ca-phytate (CaPA) and Na-phytate (NaPA) were obtained from Sigma (Catalogue 20 2003 P 9539 and P 3168, respectively).

Iron-phytate (FePA) was prepared by dissolving Na-phytate in demineralized water ar the pH was adjusted to pH= 6.3 with 5% acetic acid. and the pH was adjusted to pH = 6.3 with 0.9M sulfuric acid. Subsequently, an amount of 0.5M iron(II)sulfate-heptahydr was added until precipitation occurred. The precipitate was centrifuged and the sedim was washed with 75% water and 25% ethanol. In the second wash step the precipitat was washed with 50% water and 50% ethanol. Finally the pellet was washed twice wi 100% ethanol. Subsequently the sediment was freeze-dried. The freeze-dried FePA v analyzed for its moisture and ash content. The iron content was determined by AES/I (Atomic Emission Spectroscopy/ Inductively Coupled Plasma).

ZnPA and MgPA were prepared in the similar way as FePA, by using the correspondi metal-sulfate. Indeed, this method is suitable for all metal phytates.

Example 4:

Treatment of various metal phytates with phytase.

The activity of *Aspergillus niger* phytase (NATUPHOS™) was tested against NaPA, CaPA. The metal phytates prepared in Example 1, were treated with the liquid formulation NATUPHOS™ 5000 L (DSM, Delft, The Netherlands). The influence of th metal in the phytate source on the phytase activity (37 °C using an incubation time of minutes) was investigated with dodecasodium phytate as reference. The rate of phyt hydrolysis was determined using the analytical method described by Engelen et al., J AOAC Int. 77:760-764 (1994).

The results, expressed as FTU/g, are shown in Table 1.

Table 1: Phytase activity on different substrates.

Substrate	Activity (FTU/g)
5.1 mM sodium phytate	$3.06 * 10^5$
4.6 mM calcium phytate	$3.12 * 10^5$
5.1 mM calcium phytate	$3.07 * 10^5$
5.6 mM calcium phytate	$3.10 * 10^5$

The activity of phytase is equivalent on sodium and on calcium phytate.

Example 5:

Comparative example: mineral and phytate release without phyta

Various phytate-containing foodstuffs are commercially available. We have used Cruesli™ (Quaker Oats) as a model. The Cruesli™ was ground to a powder, to avoi inhomogeneity problems when sampling. About 20 g of ground Cruesli™ was suspe in 40 g acetate buffer (0.25 M, pH 5.5, 37 °C) or in 40 g diluted HCl (0.08 N, final pH 37 °C). The suspensions were incubated at 37°C with constant stirring for 1,5 h, an subsequently centrifuged for 20 minutes at 16000 rpm and 4°C. After centrifugation sediment and the supernatant fraction were separated, weighed and analyzed by Al ICP to determine their phosphorus, calcium, magnesium and zinc content.

The results, expressed in mg, are shown in Table 2:

Table 2: Mineral amounts in ground Cruesli™ fractions after 1,5 hours of incubation °C at different pH values.

	Phosphorus (mg)		Calcium (mg)		Magnesium (mg)		Zinc (mg)	
	super-natant	pellet	super-natant	pellet	super-natant	pellet	super-natant	Pellet
pH 2	7.12	37.8	3.03	3.02	7.49	5.51	0.158	0.132
pH 5.5	13.4	29.4	1.87	4.34	4.81	8.37	0.039	0.242

Table 2 shows that the solubility of phosphorus-containing species is higher at pH= than at pH= 2. For the metal ions, it is clear that these are more soluble at the low p

The solubility at pH= 2 may be regarded as the upper limit that may be reached by an effective phytase treatment at higher pH levels.

Example 6: Treatment of phytate-containing foodstuffs with phytas

Various phytase preparations are commercially available. We have used the liquid formulation NATUPHOS™ 5000 L (DSM, Delft, The Netherlands). Again we used gro Cruesli™ (Quaker Foods & Beverages) as model phytate-containing foodstuff. About 20 grams ground Cruesli™ was suspended in two separate lots of 40 g acetate buffer (0.25 M, pH 5.5, 37 °C). To one of the lots, 1.2 g of the phytase preparation wa added, to achieve a final activity of 100 FTU/g. The suspensions were incubated at 37°C with constant stirring for 1,5 h, and subsequently centrifuged for 20 minutes at 16000 rpm and 4°C. After centrifugation the sediment and the supernatant fraction we separated, weighed and analyzed by AES-ICP to determine their phosphorus, calciur magnesium and zinc content.

The results, expressed as the percentage of the minerals present in the supernatant fraction, are shown in Table 3:

Table 3: Mineral partitioning in ground Cruesli™ fractions after 1.5 h of incubation at 5.5 at 37 °C, with or without added phytase activity.

	Phosphorus	Calcium	Magnesium	Zinc
	% of mineral in supernatant fraction			
pH 5.5	31	31	36	14
pH 5.5 phytase 100 FTU /g	42	40	49	19

Table 3 shows that phytase treatment is effective in increasing the solubility of phosphorus and metals at pH= 5.5.

Example 7: Treatment of phytate-containing foodstuffs with lower dosage of phytase.

The procedure for this example was the same as for Example 6, but a lower final phytase activity in the incubation was used: 1 FTU/g, instead of 100. The incubation t was lowered to 1 hour.

The results, expressed as the percentage of the minerals present in the supernatant fraction, are shown in Table 4:

Table 4: Mineral partitioning in ground Cruesli™ fractions after 1 h of incubation at pH at 37 °C, with or without added phytase activity.

	Phosphorus	Calcium	Magnesium	Zinc
	% of mineral in supernatant fraction			
pH 5.5	25	27	30	13
pH 5.5 phytase 1 FTU /g	30	30	36	22

Table 4 shows that also a lower dosage of phytase is effective in increasing the solu of phosphorus and metals at pH= 5.5. By comparing the results from Example 7 with Example 6, we see that the effect of the phytase dosage is not linear, suggesting tha the accessibility of the substrate is (partly) determining the reaction rate.

Example 8:

Treatment of phytate-containing foodstuffs at pH= 2.

The procedure for this example was the same as for Example 7, but at a lower pH va and a longer incubation time. This was done to simulate the conditions extant in the stomach. We looked specifically at iron, because iron salts are believed to be particu poorly soluble at higher pH values, making breakdown in the stomach an attractive option. Iron concentrations were determined by AES-ICP. The results, expressed as percentage of the minerals present in the supernatant fraction, are shown in Table 5:

Table 5: Iron and phosphate partitioning in ground Cruesli™ fractions after 2 h of incubation at pH 2 at 37 °C, with or without added phytase activity.

	Phosphorus	Iron
	% of mineral in supernatant fraction	
pH 2	14	8
pH 2 phytase 1 FTU /g	33	14

Table 5 shows that phytase is also effective at lower pH values in increasing the solubility of phosphorus and metal ions.

Example 9:

Making a mineral-phytate-phytase preparation

Fe-phytate was prepared according to Example 3. The Fe-phytate was mixed with NATUPHOS™ 5000 G in the proportion of 9:1 (w/w), to achieve a final phytase activit 500 FTU per gram dry Fe-phytate-phytase preparation.

1 g Ca-phytate (Sigma, Catalogue 2002-2003 P 9539) was suspended in 10 g water. Subsequently, the pH was brought to pH= 2 with concentrated HCl, and the suspensi was stirred until all Ca-phytate had dissolved. Subsequently, 0.5 g of NATUPHOS™ 5000 L was added, to achieve a final activity of 250 FTU per gram of liquid Ca-phytat phytase preparation. This preparation has to be used immediately, to avoid premature breakdown of the phytate.

Example 10:

Addition of the preparation of the invention to cereal and following the action of phytase thereon

The Fe-phytate-phytase preparation described in Example 9 was used, using the Fe-phytate from Example 3 and NATUPHOS™ 5000 G (DSM, Delft, The Netherlands). 0 of this preparation was added to 20 g ground Cruesli™ (Quaker Foods & Beverages). Additional NATUPHOS™ was added to achieve a total dosage of 300 FTU per g of th fortified food. Subsquently, the food containing the metal-phytate-phytase preparatior was suspended in 40 g diluted HCl (0.08 N, final pH= 2, 37 °C), giving a final activity c 100 FTU per g suspension. The suspension was incubated at 37 °C with constant stir for 4 h, and subsequently centrifuged for 20 minutes at 16000 rpm and 4 °C. After centrifugation, the sediment and the supernatant fraction were separated, weighed ai analyzed by AES-ICP to determine their phosphorus, calcium, magnesium, zinc and content. Control suspensions without phytase activity (but with the FePA), and withou both FePA and phytase underwent the same procedure.

The increased solubility of metal ions shows that phytase is also effective in foods enriched with essential cations bound to phytate.

Example 11:

Phytase stability in soy sauce

Soy sauce (Kikkoman, Sappemeer, The Netherlands) was used as model foodstuff · determine the stability of NATUPHOS™ (DSM, Delft, The Netherlands). NATUPHOS™ 5000 L was added to Soya sauce to reach a final concentration of 5% (w/w). This suspension was diluted 100-fold in 0.25M acetate buffer (pH= 4.8, which the endogenous pH-value of the Soy sauce), and incubated at 20 °C and 35 °C for 4 weeks. The phytase activity of different time samples was determined using the analytical method described by Engelen et al., J. AOAC Int. 77:760-764 (1994). The results, expressed in mg, are shown in Table 6:

Table 6: Phytase stability in soya sauce (1% w/w) at different temperature.

	Incubation	
	20 °C	35 °C
Time (weeks)	Activity (FTU/g)	Activity (FTU/g)
0	2,63	2,63
2	2,59	2,35
4	2,59	2,21

It is clear that phytase is sursprisingly stable in this condiment, even at 35 °C

Example 12:

Phytase stability in foodstuffs with and without phytate

The stability of phytase in other foodstuffs was also determined. NATUPHOS™ 5000L, a liquid preparation containing 5000 FTU / g, was added to Oy Sauce (Lee Kum Kee, Hong Kong, China), Fish Sauce (Pantainorasingh manufactur Thailand), Tomato Ketchup (H.J. Heinz, Elst, the Netherlands), Chilli Sauce (Flower Brand, Ho Chi Minh City, Vietnam) and Soy sauce (Kikkoman, Sappemeer, the Netherlands) to a final concentration of 1% (w/w) phytase in the condiment. The suspensions were incubated at 20 °C and 35 °C for 3 months.

NATUPHOS™ 5000G, a granulated preparation containing 5000 FTU / g, was added the gritting cheese "Formaggio da Pasta" (Grozette, the Netherlands) to a final concentration of 1% (w/w) and incubated at 4°C and 20°C for 3 months.
The phytase activity of different time samples was determined using the analytical method described by Engelen et al., J. AOAC Int. 77:760-764 (1994).
It was found that phytase was surprisingly stable after three months of storage in all o these condiments and foods.

Example 13:

Stability of phytase after pasteurization in soymilk

NATUPHOS™ 5000L was added to soymilk (Alpro, Izegem, Belgium) to a final concentration of 10 FTU/g. The soymilk was pasteurized during 20 seconds at 85°C. After addition of NATUPHOS™ to the soy milk, samples were taken before and after pasteurization to determine the phytase activity according to the analytical method described by Engelen et al., J. AOAC Int. 77:760-764 (1994).
It was found that 80% of the initial phytase activity was retained after this pasteurizati treatment.

Example 14:

Method to determine the amount of essential cation bound to phyta

The prepared FePA as described in Example 3 was suspended either at pH 2 or at pl 5,5 in a concentration of 35.8 g per liter. After sedimentation, the supernatant fraction both suspensions was analysed by AES/ICP. It was found that more than 90% of the was retained in the sedimented fraction at both pH values.
Subsequently, the suspension of pH= 2 was centrifuged 10 minutes at 6000 rpm at rc temperature. The pellet was dissolved in 15 g concentrated chloric acid and the solut was analyzed by AES/ICP. Now, essentially all Fe could be retrieved in the soluble fraction.
This procedure was repeated with iron phosphate. This salt was prepared in the sam way as the preparation of the essential cation phytates: the cation was dissolved as i sulfate, and subsequently precipitated with a basic solution of sodium phosphate. Th precipitate was collected and washed. When the iron phosphate was incubated at pH

and pH= 5.5, it was found that more than 90% of the Fe was retained in the sedime fraction at pH= 5.5, but that less than 10% was retained at pH= 2.
This provides a clear difference in behaviour for essential cations bound to phytate, compared to their other poorly soluble salts

Claims

1. Preparation comprising an active phytase, a phytate and an essential cation, characterized in that at least part of the essential cation is bound to phytate.

2. Preparation according to claim 1, characterized in that, the preparation compri more than 1 FTU phytase per gram phytate, more than 1 g essential cation bound to phytate and less than 99 g of phytate per 100 g of essential cation bound to phytate.

3. Preparation according to claim 2, characterized in that, the preparation compri between 1 and 100 FTU phytase per gram phytate, between 1 and 50 g essen cation bound to phytate and between 50 and 99 g of phytate per 100g of essential cation bound to phytate.

4. Preparation according to any one of claim 1 to 3, characterized in that when th preparation is present in the intestinal tract, essential cations are released fron the phytate.

5. Preparation according to any one of claim 1 to 4, characterized in that the essential cation is selected from the group consisting of calcium, zinc, iron, magnesium, cobalt, molybdenum, manganese, chromium, copper or a combination thereof.

6. Preparation according to any one of claims 1 to 5, characterized in that an additional component is present in the preparation, said component being selected from the group consisting of a chelating agent, an antioxidant.

7. Method for making the preparation according to any one of claims 1 to 6.

8. Use of the preparation according to any one of claims 1 to 6, for making a fortified food product.

9. Food product comprising the preparation of any one of claims 1 to 6, characterized in that the food product is selected from the group consisting muesli, flour, rice, pasta, cereal bar, bread, cake, pastry, cracker, cow milk, soymilk, cheese, yogurts, milk shakes, creams, desserts, condiment.

10. Use of the preparation of any one of claims 1 to 6 or of the food product of c 9 for increasing the availability of an essential cation for humans.

11. Condiment comprising an active phytase.

12. Condiment according to claim 11, characterized in that the condiment is supplemented with an essential cation.

13. Condiment according to claim 11 or 12, characterized in that the condiment i selected from the group consisting of soy sauce, tomato sauce or flavor enhancers such as curry powder.

14. Soymilk comprising an active phytase.

15. Soymilk according to claim 14, characterized in that the condiment is supplemented with an essential cation.

16. Use of condiment according to any one of claim 11 to 13 as a delivery systen phytase in human consumption.

17. Use of soymilk according to claim 14 or 15 as a delivery system for phytase i human consumption.

Abstract

The invention relates to a preparation comprising an active phytase, a phytate and an essential cation, said preparation being characterized in that at least part of the essent cation is bound to phytate. The preparation may comprise between 1 and 100 FTU phytase per gram phytate, between 1 and 50 g essential cation bound to phytate and between 50 and 99 g of phytate per 100g of essential cation bound to phytate. The essential cations are selected from the group consisting of calcium, zinc, iron, magnesium, cobalt, molybdenum, manganese, chromium, copper or a combination thereof. The preparation may comprise an additional component such as a chelating agent and/or an antioxidant. The invention also relates to a method for making the preparation of the invention and to its use for making a fortified food product. The invention also relates to a condiment, a cereal product or a soymilk comprising an acti phytase.